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Magic Spots Cast a Spell on DNA Primase

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The bacterial signaling molecules ppGpp and pppGpp regulate transcription initiation in response to starvation by altering RNA polymerase activity. In this issue, Wang et al. (2007) show that (p)ppGpp also inhibits DNA replication elongation by interfering with DNA primase activity. Halting replication may help cells to maintain genomic integrity during periods of transient nutrient limitation.

Bacteria lead a feast or famine existence. Not surprisingly, they have elegant mechanisms to ensure survival in times of nutritional stress. For example, it has been known for half a century that starvation of various kinds triggers the "stringent response," which dramatically alters the genome-wide transcription profile (reviewed by Cashel et al., 1996). The stringent response has been best characterized in *Escherichia coli*, where amino acid starvation leads to changes in gene expression including inhibition of promoters for ribosomal and most transfer RNA (tRNA) operons and stimulation of promoters for many amino acid biosynthesis operons. The primary signaling molecules for these responses are the guanosine nucleotides pppGpp and ppGpp, which bind directly to RNA polymerase. These unusual nucleotides are collectively referred to as (p)ppGpp or "magic spots I and II" from their original identification on thin layer chromatograms by Mike Cashel in the late 1960s.

A variety of reports have hinted that (p)ppGpp's effects extend beyond transcription (see Wang et al., 2007 for references). For example, amino acid starvation has been reported to cause arrest of DNA duplication in *Bacillis subtilis* by stalling the replication machinery at positions on the chromosome called LSTer and RSTer (*l*eft and *r*ight stringent *t*erminator) sites (Autret et al., 1999). To test whether nutritional stress terminates replication in a locus-specific manner, Wang and colleagues followed the progress of DNA replication in synchronized *B. subtilis* cultures using a time-resolved genomic microarray assay. The progress of the bidirectional replication forks could be determined with remarkable precision by visualizing the increase in DNA from one to two genome equivalents. Furthermore, the positions where replication forks halted in response to amino acid starvation could be easily identified in this assay. Contrary to the LSTer/RSTer site model, replication fork stalling was not determined by specified positions in the genome, but rather the position of fork stalling was determined by the interval of time that the forks were allowed to progress following initiation of replication. If the forks had already moved past the LSTer/RSTer sites when starvation was induced, they were still blocked from progressing further. Inhibition of replication in nonsynchronized cultures and the absence of a requirement for the replication termination protein Rtp were consistent with the interpretation that replication elongation was arrested not at a specific site but throughout the genome in response to nutrient stress.

How then does amino acid starvation lead to the termination of DNA replication? It has long been known

that (p)ppGpp is induced by amino acid starvation in *B. subtilis*, as it is in *E. coli*. Wang and coworkers knew that (p)ppGpp exerted an effect on the DNA replication machinery, directly or indirectly, because replication was not blocked in a mutant lacking *relA* (Autret et al., 1999), the gene responsible for (p)ppGpp synthesis throughout the bacterial kingdom and even in chloroplasts. Ultimately, they showed that (p)ppGpp targeted replication directly in vitro by inhibiting DNA primase, a specialized RNA polymerase that primes DNA replication (reviewed by Frick and Richardson, 2001). Given its central roles in DNA replication initiation and elongation, the cell makes a shrewd decision in choosing to target primase.

As both primase and RNA polymerase are DNA-dependent RNA polymerases, one might think that identification of a binding site(s) for (p)ppGpp on RNA polymerase would provide clues as to the location(s) of its target(s) on primase. However, exactly where (p)ppGpp binds to RNA polymerase and how this binding alters transcription initiation remain unresolved. A binding site for ppGpp on *Thermus thermophilus* RNA polymerase was defined at the atomic level (Artsimovitch et al., 2004), but it is questionable whether *T. thermophilus* RNA polymerase is actually inhibited by (p)ppGpp in vivo (Kasai et al., 2006). Furthermore, (p)ppGpp works efficiently on *E. coli* RNA polymerase only in conjunction with a cofactor, the transcription factor DksA (Paul et al., 2004), which binds in the secondary channel of the enzyme (Perederina et al., 2004). Not only does DksA appear to be absent in *B. subtilis*, but (p)ppGpp appears to function on RNA polymerase only indirectly in this bacterium, at least in part by reducing the concentration of GTP (Krasny and Gourse, 2004). Thus, it would be imprudent at this time to suppose that one could identify the (p)ppGpp binding site on primase based on current information about a homologous site on RNA polymerase.

Nevertheless, several features of primases make it tempting to speculate that inhibition of replication elongation could result from binding of (p)ppGpp at the primase active site. The primase active site comprises a conserved metal binding cleft with extended electropositive regions that form the likely binding sites for template and nucleotide triphosphates (NTPs) (Keck et al., 2000). The active site cleft of primase is significantly more exposed than those of other polymerases, and the protein has a remarkably high misincorporation rate of $\sim$1 in 10 (Frick and Richardson, 2001), implying that unusual nucleosides such as (p)ppGpp could possibly bind the active site. Indeed, several nucleoside-based inhibitors of bacterial primases have been identified. The precise structural and kinetic mechanisms by which (p)ppGpp inhibits primase remain to be determined.

Whatever the mechanism of inhibition, we can nevertheless speculate about the rationale for control of primase activity and thereby the control of replication elongation by (p)ppGpp. Wang et al. demonstrate that *relA*-dependent inhibition of replication elongation does not recruit the SOS response protein RecA to stalled forks. These results suggest that replication forks are not disrupted by (p)ppGpp, or at least that significant sections of single-stranded DNA (generated at damaged replication forks and bound by RecA, leading to the SOS response) are not generated at the stalled forks. In addition, *recA* is not required for recovery following the inhibition of replication elongation, further indicating that the SOS response is not induced upon amino acid starvation.

The authors therefore propose that primase inhibition by (p)ppGpp serves to maintain genome integrity under conditions where the synthesis of the substrates of replication, dNTPs, are depleted by a temporary stoppage of translation, but there is no damage to the replication fork. Replication forks could remain in suspended animation under these conditions, awaiting the resumption of protein synthesis and replenishment of dNTP pools. Concurrent reduction of ribosome synthesis and induction of amino acid biosynthesis would allow recovery of amino acid pools, resumption of charging of tRNAs, restoration of (p)ppGpp to basal levels, and relief from the temporary arrest of replication caused by starvation. Many aspects of this model remain to be tested, including whether (p)ppGpp inhibits primase in other bacteria. If so, primase inhibition by (p)ppGpp provides an elegant link in bacteria between the regulation of DNA replication and nutrient availability, a link that likely evolved very early in the history of life on earth.

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